Data Evaluation Report on the acute toxicity of Dithane/RH-7281 80 WP [(10:1) Containing 67.8% Mancozeb and 7.21% Zoxamide] to Rainbow Trout (*Oncorhynchus mykiss*)

PMRA Submission Number {.....} EPA MRID Number 46056401

Data Requirement:	PMRA DATA CODE	{.....}
	EPA DP Barcode	D298722
	OECD Data Point	
	EPA MRID	46056401
	EPA Guideline	OCSPP 850.1075

Test material: Dithane/RH-7281 80 WP (formulated product) **Purity:** 71.00% mancozeb
7.09% zoxamide

Common name: Zoxamide, mancozeb
Chemical name: IUPAC: Not reported
CAS name: Not reported
CAS No.: Not reported
Synonyms: Dithane (mancozeb); RH-7281 (zoxamide); RH-117,281 (zoxamide)

Primary Reviewer: Christie E. Padova
Staff Scientist, Dynamac Corporation

Signature:
Date: 5/31/04

QC Reviewer: Teri S. Myers, Ph.D.
Staff Scientist, Dynamac Corporation

Signature:
Date: 6/25/04

Primary Reviewer: Robin Sternberg
EAP/OCSPP/OPP/EFED/ERB1

Date:
Digitally signed by Sternberg, Robin
DN: cn=Sternberg, Robin, email=sternberg.robin@epa.gov
Date: 2014.10.15 11:16:32 -04'00'

Secondary Reviewer(s): Freeborn G. Jewett
EAP/OCSPP/OPP/EFED/ERB1

Date:
FREEBORN JEWETT
2014.10.15 10:56:20 -04'00'

Reference/Submission No.:

Company Code:
Active Code:
EPA PC Code: 101702 (zoxamide) and 014504 (mancozeb)

Date Evaluation Completed: 3/31/14

CITATION: Dickson, J., and B. Knight. 1998. Dithane/RH-7281 80WP: Determination of Acute Toxicity (LC_{50}) to Rainbow Trout (96 h, Continuous Flow). Unpublished study performed by Inveresk Research, Tranent EH33 2NE, Scotland, and submitted by Rohm & Haas Company, Spring House, PA. Laboratory Report No. 16697. Study initiated April 10, 1998 and completed December 7, 1998.

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EXECUTIVE SUMMARY:

In a 96-hour acute toxicity study, Rainbow trout (*Oncorhynchus mykiss*) were exposed under flow-through conditions to Dithane/RH-7281 80WP (10:1) (a formulated product containing 71.00% mancozeb and 7.09% zoxamide) at nominal concentrations of 0 (negative control), 0.025, 0.05, 0.1, 0.2, and 0.4 mg/L formulated product. Whole product mean-measured concentrations were <0.04, <0.004 [<LOQ (for mancozeb, zoxamide), negative control], 0.087, 0.008, 0.092, 0.175, and 0.451 mg/L formulated product. Mean-measured concentrations of zoxamide were <0.004 [<LOQ], 0.002[<LOQ], 0.004, 0.006, 0.012, 0.022 mg a.i./L. Mean-measured concentrations for mancozeb were <0.04 [<LOQ], 0.063, 0.002, 0.063, 0.120, and 0.520 mg a.i./L.

After 96 hours of exposure, cumulative mortality was 0% in the negative control group, and 0, 0, 10, 60, and 100% in the mean-measured 0.087, 0.008, 0.092, 0.175, and 0.451 mg/L formulated product test groups, respectively. The 96-hour LC_{50} (with 95% C.I.) was 0.159 (0.129-0.232) mg/L formulated product, which **categorizes Dithane/RH-7281 80WP (10:1) as highly toxic to Rainbow trout (*Oncorhynchus mykiss*)** on an acute toxicity basis. Dark coloration, lethargy, and/or near the bottom of the tank were observed in fish from the ≥0.092 mg/L formulated product test levels. Effects were first observed after 16 hours of exposure at the 0.451 mg/L level. The NOAEC and LOAEC, based on sub-lethal effects, were 0.087 and 0.092 mg/L formulated product, respectively.

This study is scientifically sound and satisfies the guideline requirements for an acute toxicity study with rainbow trout using a formulated product. This study is classified as **Acceptable**.

Results Synopsis

Test Organism Size/Age (mean Weight or Length):	Initial age and size were not reported. Wet weight and lengths of all surviving fish at study termination were 42-57 mm and 1.027-2.869 g, respectively (Table 3, p. 18).
Test Type (Flow-through, Static, Static Renewal):	Flow-through

96-Hour (formulated product)
LC_{50}: 0.159 mg/L 95% C.I: 0.129-0.232 mg/L
Slope: 5.82 95% C.I.: 1.87-9.76
NOAEC: 0.087 mg/L
LOAEC: 0.092 mg/L

96-Hour (zoxamide)
LC_{50}: 0.010 mg/L 95% C.I: 0.008-0.013 mg/L
Slope: N/A
NOAEC: 0.002 mg/L
LOAEC: 0.006 mg/L

96-Hour (mancozeb)
LC_{50}: 0.107 mg/L 95% C.I: 0.079-0.260 mg/L
Slope: 5.50 95% C.I.: 0.88-10.1
NOAEC: 0.003 mg/L
LOAEC: 0.0063 mg/L

Endpoints affected: Mortality and sub-lethal effects (more sensitive)

Data Evaluation Report on the acute toxicity of Dithane/RH-7281 80 WP [(10:1) Containing 67.8% Mancozeb and 7.21% Zoxamide] to Rainbow Trout (*Oncorhynchus mykiss*)

I. MATERIALS AND METHODS

GUIDELINES FOLLOWED: The study protocol was based on procedures outlined in OECD Guideline 203 (1992); TSCA No. 139, IBAMA, Brazil (1994); U.S. EPA FIFRA Subdivision E, Series 72-1 (1982); U.S. EPA Standard Evaluation Procedure; and ASTM Standard E729-88a. Deviations from U.S. EPA OCSPP 850.1075 included:

- The initial age, weight, and length of the fish were not specified. Individual wet fish weight and lengths were determined from all surviving fish at study termination.
- The pre-test health (including mortality) of the fish during the acclimation period was not reported.
- The dilution water was charcoal-filtered de-chlorinated tap water, which is not recommended for use in aquatic toxicity studies.
- The hardness (72-78 mg/L as $CaCO_3$) was higher than recommended (40-48 mg/L as $CaCO_3$).
- The pH range (6.8-7.2) was slightly lower than recommended (7.2-7.6).
- Results of analysis of the dilution water for TOC, particulate matter, metals, pesticides, and chlorine were not provided.
- The temperature range (14.5-15.0°C) was slightly higher than recommended for cold water species (12°C).
- Mean-measured concentrations were determined by analytical determination at 0 and 96 hours of both active ingredients, mancozeb (Dithane) and zoxamide (RH-7,281). Mancozeb recoveries were highly variable at the nominal 0.1 mg/L formulated product level (reviewer-calculated high-low ratio of 3.2), notably lower than expected at the nominal 0.05 mg/L test level, and notably higher than expected at the nominal 0.025 mg/L level. Back-calculated mean-measured concentrations of total formulated product thus reflected these fluctuations in mancozeb recoveries. This variability did not impact the ability to derive sound toxicity estimates from this study, so it did not affect the study classification.
- The biomass loading rate was not reported.

These deviations did not affect the validity or acceptability of the study.

COMPLIANCE: Signed and dated GLP, Confidentiality, and Quality Assurance statements were provided. This study was conducted according to OECD GLP standards (p. 3).

A. MATERIALS:

1. Test Material Dithane/RH-7281 80WP (10:1) (formulated product)

Description: Yellow powder

Lot No./Batch No. : TB-0332 (lot no.)

Purity: 71.00% mancozeb (Dithane) and 7.09% zoxamide (RH-117,281; RH-7281)

Stability of Compound Under Test Conditions: The stability of the formulated product in the dilution water during the course of the study was assessed at 0 and 96 hours by analytical determination of both active ingredients, mancozeb (Dithane) and zoxamide (RH-7,281). Zoxamide recoveries were relatively constant at both sampling intervals. However, mancozeb recoveries were highly variably at the nominal 0.1 mg/L formulated product level (reviewer-calculated high-low ratio of 3.2). Since high variability was only observed for one analyte at one test level, this deviation was considered minor by the reviewer, and the test substance was considered stable under the conditions of the study.

Storage conditions of test chemical: Ambient temperature in the dark

OECD requires water solubility, stability in water and light, pK_a, P_{ow}, and vapor pressure of the test compound. OECD requirements were not reported.

2. Test organism:

Species: Rainbow trout (*Oncorhynchus mykiss*)

Age at test initiation: Not reported

Weight at test initiation: Not provided; the wet weights of all surviving fish measured at test termination ranged from 1.027-2.869 g.

Length at test initiation: Not provided; the lengths of all surviving fish measured at test termination ranged from 42-57 mm.

Source: Selcoth Fish Farm, Moffat (presumably Scotland)

B. STUDY DESIGN:

1. Experimental Conditions

a. Range-finding Study: A 96-hour preliminary range-finding experiment was conducted under flow-through conditions. Five trout were tested per level, at nominal concentrations of 0 (negative control), 0.004, 0.04, 0.4, and 4 mg/L formulated product (p. 10). Mortality was 100% after 24 hours at the 4 mg/L formulated product level and after 48 hours at the 0.4 mg/L level (p. 13). No other mortality was observed. After 72 hours, fish at the nominal 0.04 mg/L formulated product level appeared dark in coloration.

b. Definitive Study:

Table 1: Experimental Parameters

Parameter	**Details**	**Remarks**
		Criteria
Acclimation period:	≥12 days	Upon receipt, fish were reported to be in good health and free from any apparent malformation.
Conditions: (same as test or not)	Same as test	
Feeding:	Suitable standard diet (not otherwise specified), except during the 24 hours prior to testing and throughout the duration of the test	*EPA requires: minimum 14 days; no feeding during test OECD requires minimum of 12 days.*
Health: (any mortality observed)	All fish were in good health and free from any apparent malformation. Health (including mortality) during acclimation was not reported.	
Duration of the test	96 hours	
		EPA/OECD requires: 96 hours
Test condition static/flow through	Flow-through	The diluter system was calibrated prior to testing and checked daily during testing. Each test chamber received approximately 5.0-5.2 volume additions per day.
Type of dilution system- for flow through method.	Continuous-flow diluter	
Renewal rate for static renewal	N/A	*EPA: Must provide reproducible supply of toxicant, with a consistent flow rate of 5-10 vol/24 hours, and meter systems calibrated before study and checked twice daily during test period*
Aeration, if any	None reported	
		EPA requires: no aeration; OECD permits aeration

Parameter	**Details**	**Remarks** **Criteria**
Test vessel Material: (glass/stainless steel) Size: Fill volume:	 Moulded glass with perspex lids 25 L 15 L	Vessels were covered with perspex lids. *EPA requires: Size 19 L (5 gal) or 30 x 60 x 30 cm* *Fill volume: 15-30 L of solution*
Source of dilution water	Charcoal-filtered de-chlorinated tap water	De-chlorinated tap water is not recommended for use in aquatic toxicity studies. *EPA 1975; Soft reconstituted water or water from a natural source, **not** dechlorinated tap water;* *OECD permits dechlorinated tap water.*
Water parameters: Hardness pH Dissolved oxygen Total Organic Carbon Particulate Matter Metals Pesticides Chlorine Temperature Intervals of water quality measurement	 72-78 mg $CaCO_3$ /L 6.8-7.2 87-92% saturation Not reported Not reported Not reported Not reported Not reported 14.5-15.0°C The DO, pH, and temperature were determined in each test chamber at 0, 24, 48, 72, and 96 hours.	The hardness was higher than recommended. The pH range was lower than recommended. ***Hardness and pH*** *EPA requires hardness of 40-48 mg/L as $CaCO_3$ and pH of 7.2-7.6. OECD allows hardness of 10-250 mg/L as $CaCO_3$ and pH between 6 and 8.5.* ***Dissolved Oxygen*** *Renewal: ≥60% during 1st 48 hrs and ≥ 40% during 2nd 48 hrs* *Flow-through: ≥60% through out test. OECD requires at least 80% saturation value.* ***Temperature*** *EPA requires 12℃ for coldwater species and 17-22℃ for warmwater*

Parameter	Details	Remarks
		Criteria
		species. OECD requires range of 21 - 25℃ for bluegill and 13-17℃ for rainbow trout. ***EPA water quality*** *measured at beginning of test and every 48 hours*
<u>Concentration of test material:</u> nominal: measured:	0 (negative control), 0.025, 0.05, 0.1, 0.2, and 0.4 mg/L formulated product <0.04, 0.004 [<LOQ (for mancozeb, zoxamide), negative control], 0.087, 0.008, 0.092, 0.175, and 0.451 mg/L formulated product <0.004 [<LOQ, negative control], 0.002[<LOQ], 0.004, 0.006, 0.012, 0.022 mg a.i./L zoxamide <0.04 [<LOQ, negative control], 0.063, 0.002, 0.063, 0.120, and 0.520 mg a.i./L mancozeb	Mean-measured concentrations are provided in Table 1, p. 16, and were determined by analytical determination at 0 and 96 hours of both active ingredients, mancozeb (Dithane) and zoxamide (RH-7,281). Zoxamide recoveries were relatively constant at both sampling intervals, and correlated well with expected nominal concentrations (although three out of four results were <LOQ at the two lowest test levels). However, mancozeb recoveries were highly variably at the nominal 0.1 mg/L formulated product level (reviewer-calculated high-low ratio of 3.2), notably lower than expected at the nominal 0.05 mg/L test level, and notably higher than expected at the nominal 0.025 mg/L level. Back-calculated mean-measured concentrations of total formulated product thus reflected these fluctuations in mancozeb recoveries.
		EPA/OECD requires: Control and five treatment levels. Each conc. should be 60% of the next highest conc., and should be in a geometric series
Solvent (type, percentage, if used)	None used	
		EPA requires: Not to exceed 0.5 mL/L for static tests or 0.1 mL/L for flow-through tests; OECD requires solvent , exceed 100 mg/L.
<u>Number of fish/replicates</u>: negative control:	One replicate containing 10 fish	

Parameter	**Details**	**Remarks**
		Criteria
solvent control: treated:	N/A One replicate containing 10 fish	*EPA: ≥10/concentration; OECD requires at least 7 fish/concentration*
Biomass loading rate	Not reported	
		Static: ≤0.8 g/L at ≤17°C, ≤0.5 g/L at >17°C; flow-through: ≤1 g/L/day; OECD requires maximum of 1 g fish/L for static and semi-static with higher rates accepted for flow-through
Lighting	16-hours light/8-hours dark	Light intensity was approximately 602 lux (artificial daylight fluorescent tubes).
		EPA requires: 16 hours light/8 hours dark); OECD requires 12 -16 hours photoperiod.
Feeding	Fish were not fed during testing.	
		EPA/OECD requires: No feeding during the study
Recovery of chemical Level of Quantitation Level of Detection	83.3-111% of nominal (RH-7281 at 0 hours) 0.004 mg/L zoxamide (RH-7281) 0.04 mg/L mancozeb (Dithane) Not reported	Percent recoveries were reviewer-calculated for RH-7281 from measured 0-hour concentrations versus expected nominal concentrations for this analyte (using only concentrations where the analyte was >LOQ; Table 1, p. 16).
Positive control {if used, indicate the chemical and concentrations}	N/A	
Other parameters, if any	N/A	

2. Observations:

Table 2: Observations

Criteria	**Details**	**Remarks/Criteria**
Parameters measured including the	Mortality and sub-lethal effects	

sub-lethal effects/toxicity symptoms		
Observation intervals	0, 1, 3, 6, 24, 48, 72, and 96 hours	
		EPA/OECD requires: minimally every 24 hours
Were raw data included?	Yes, sufficient	
Other observations, if any	N/A	

II. RESULTS AND DISCUSSION:

A. MORTALITY:

After 96 hours of exposure, cumulative mortality was 0% in the negative control group, and 0, 0, 10, 60, and 100% in the mean-measured 0.087, 0.008, 0.092, 0.175, and 0.451 mg/L formulated product test groups, respectively (Table 2, p. 17). The 96-hour LC_{50} (with 95% C.I.) was 0.159 (0.129-0.232) mg/L formulated product (p. 14).

Table 3: Effect of Dithane/RH-7281 80WP (10:1) (containing 71.00% mancozeb and 7.09% zoxamide) on mortality of Rainbow trout (*Oncorhynchus mykiss*).

Treatment, mg/L formulated product, measured and (nominal conc.)	No. of fish at start of study	Observation Period					
		24 Hours		72 Hours		96 Hours	
		No Dead	% mortality	No Dead	% mortality	No Dead	% mortality
Negative control	10	0	0	0	0	0	0
0.087[2] (0.025)	10	0	0	0[1]	0	0	0
0.008 (0.05)	10	0	0	0	0	0	0
0.092 (0.1)	10	0	0	1	10	1	10
0.175 (0.2)	10	0	0	4	40	6	60
0.451 (0.4)	10	0	0	10	100	10	100
NOAEC (mortality)	Not reported						
LC_{50} (95% C.I.)	0.159 (0.129-0.232) mg/L formulated product						
Positive control, if used mortality: LC_{50}:	N/A	N/A	N/A	N/A	N/A	N/A	N/A

[1] One fish jumped out of tank and was found dead on bench.

[2] Note that this mean-measured value was higher than the measured value of the next higher treatment level.

B. NON-LETHAL TOXICITY ENDPOINTS:

Dark coloration, lethargy, and/or near the bottom of the tank were observed in fish from the ≥0.092 mg/L formulated product test levels (p. 14). Effects were first observed after 16 hours of exposure at the 0.451 mg/L level. The NOAEC and LOAEC, based on sub-lethal effects, were 0.087 and 0.092 mg/L formulated product, respectively.

Table 4. Sub-lethal effects of Dithane/RH-7281 80WP (10:1) (containing 71.00% mancozeb and 7.09% zoxamide) on Rainbow Trout (*Oncorhynchus mykiss*).

Treatment, mg/L formulated product, measured and (nominal conc.)	Observation Period				
	16 Hours	24 hours	48 Hours	72 Hours	96 Hours
	% affected	% affected	% affected	% affected	% affected
Negative control	AN	AN	AN	AN	AN
0.087 (0.025)	AN	AN	AN	AN	AN
0.008 (0.05)	AN	AN	AN	AN	AN
0.092 (0.1)	AN	AN	100% - Slightly dark coloration	100% - Slightly dark coloration	100% - Dark coloration 100% - Slightly lethargic
0.175 (0.2)	AN	AN	100% - Dark coloration 100% - Lethargic	100% - Dark coloration 100% - Lethargic 100% - Near base of tank	100% - Dark coloration 100% - Lethargic 100% - Near base of tank
0.451 (0.4)	100% - Dark coloration	100% - Dark coloration 100% - Lethargic	100% - Dark coloration 100% - Lethargic	—	—
NOAEC (sub-lethal)	0.087 mg/L formulated product				
LOAEC (sub-lethal)	0.092 mg/L formulated product				
EC_{50}	Not determined				

AN - All surviving fish appeared normal.
— Complete mortality.

C. REPORTED STATISTICS:

The 96-hour LC_{50} value with 95% confidence limits was calculated using probit analysis (Finney, 1971). NOAEC values were based on empirical observation. The results were based on mean-measured concentrations of the formulated product.

96-Hour
LC_{50}: 0.159 mg/L formulated product 95% C.I: 0.129-0.232 mg/L formulated product
NOAEC: 0.087 mg/L formulated product
LOAEC: 0.092 mg/L formulated product
Endpoints affected: Mortality and sub-lethal effects

D. VERIFICATION OF STATISTICAL RESULTS:

The 96-hour LC_{50} value was estimated based on concentrations for the formulated product, the active ingredient zoxamide, and the active ingredient mancozeb. The estimates based on the formulated product and mancozeb were derived using the Probit method, while the zoxamide estimate was calculated using the moving average method. These analyses were conducted using TOXANAL statistical software. The NOAEC and LOAEC values were determined visually based on sublethal effects. The lowest treatment concentration was omitted from the LC_{50} analysis for the formulated product and mancozeb concentrations because this measured concentration was higher than the measured concentration for the next higher level.

96-Hour (formulated product)
LC_{50}: 0.156 mg/L 95% C.I: 0.116-0.233 mg/L
Slope: 5.82 95% C.I.: 1.87-9.76
NOAEC: 0.087 mg/L
LOAEC: 0.092 mg/L

96-Hour (zoxamide)
LC_{50}: 0.010 mg/L 95% C.I: 0.008-0.013 mg/L
Slope: N/A
NOAEC: 0.002 mg/L
LOAEC: 0.006 mg/L

96-Hour (mancozeb)
LC_{50}: 0.107 mg/L 95% C.I: 0.079-0.260 mg/L
Slope: 5.50 95% C.I.: 0.88-10.1
NOAEC: 0.003 mg/L
LOAEC: 0.0063 mg/L

Endpoints affected: Mortality and sub-lethal effects (more sensitive)

E. STUDY DEFICIENCIES:

This study is scientifically sound. Mean-measured concentrations are provided in Table 1, p. 16, and were determined by analytical determination at 0 and 96 hours of both active ingredients, mancozeb (Dithane) and zoxamide (RH-7,281). Zoxamide recoveries were relatively constant at both sampling intervals, and correlated well with expected nominal concentrations (although three out of four results were <LOQ at the two lowest test levels). However, mancozeb recoveries were highly variably at the nominal 0.1 mg/L formulated product level (reviewer-calculated high-low ratio of 3.2), notably lower than expected at the nominal 0.05 mg/L test level, and notably higher than expected at the nominal 0.025 mg/L level. Back-calculated mean-measured concentrations of total formulated product thus reflected these fluctuations in mancozeb recoveries. However, since variability was only excessive in a

single test level (the nominal 0.1 mg/L formulated product level) for only one of the two analytes, this deviation is considered minor by the reviewer, and this study is accepted as a best effort by the laboratory.

F. REVIEWER'S COMMENTS:

The reviewer's conclusions were similar to those of the study author. The study author's estimate for the formula product LC_{50} is provided in the Executive Summary and Conclusions sections; this estimate is slightly higher than the reviewer's estimate, but it was associated with a narrower 95% confidence interval.

Higher than expected concentrations of mancozeb were observed during analysis of the nominal 0.025 mg/L formulated product test solutions at both 0 and 96 hours (p. 13 and Table 1, p. 16). The study authors reported that the reason for these high concentrations are unknown, and hypothesized that microparticulate test material may have been present in the test tank which may have been inadvertently sampled for analysis. Furthermore, lower than expected concentrations of mancozeb were observed during analysis of the nominal 0.05 mg/L formulated product test solutions at both 0 and 96 hours. The study authors reported that these low recoveries may ben due to the proximity of the nominal concentration of mancozeb (Dithane) to its LOQ (0.04 mg/L a.i.).

The study authors reported that since the measured concentrations of both mancozeb and zoxamide lie close to or below the LOQ for the nominal 0.025 and 0.05 mg/L formulated product test levels, that the back-calculated mean-measured concentrations for the total formulated product should be treated with caution (p. 13). It was also noted that these values would not significantly affect the calculated LC_{50} value since no treatment-related mortality occurred at these levels. The reviewer agrees with this statement and concludes that the analytical variability at these levels did not impact the ability to derive sound toxicity estimates from this study, so it did not affect the study classification.

All test solutions appeared clear and colorless throughout the test (p. 8).

G. CONCLUSIONS:

This study is scientifically sound and satisfies the guideline requirements for an acute toxicity study with freshwater fish, cold water species using a formulated product. This study is classified as acceptable. The 96-hour LC_{50} (with 95% C.I.) was 0.159 (0.129-0.232) mg/L formulated product, which categorizes Dithane/RH-7281 80WP (10:1) (containing 71.00% mancozeb and 7.09% zoxamide) as highly toxic to rainbow trout (*Oncorhynchus mykiss*) on an acute toxicity basis. The NOAEC (for mortality and sub-lethal effects) was 0.087 mg/L formulated product.

96-Hour (formulated product)
LC_{50}: 0.159 mg/L 95% C.I: 0.129-0.232 mg/L
Slope: 5.82 95% C.I.: 1.87-9.76
NOAEC: 0.087 mg/L
LOAEC: 0.092 mg/L

96-Hour (zoxamide)
LC_{50}: 0.010 mg/L 95% C.I: 0.008-0.013 mg/L
Slope: N/A

NOAEC: 0.002 mg/L
LOAEC: 0.006 mg/L

96-Hour (mancozeb)
LC_{50}: 0.107 mg/L 95% C.I: 0.079-0.260 mg/L
Slope: 5.50 95% C.I.: 0.88-10.1
NOAEC: 0.003 mg/L
LOAEC: 0.063 mg/L

Endpoints affected: Mortality and sub-lethal effects (more sensitive)

III. REFERENCES:

Finney, D.J. 1971. "Probit Analysis", 3rd ed. Cambridge University Press.

Fisher, R.E. 1950. "Statistical Methods for Research Workers". 21.02. Oliver and Boyd. Edinburgh.

Organization for Economic Cooperation and Development. 1992. OECD Guidelines for Testing of Chemicals, Test Guideline 203, "Fish, Acute Toxicity Test".

Official Journal of the European Communities. 1992. L383A, 29 December 1992, Annex to Commission Directive 92/69/EEC, Method C.1., "Acute Toxicity for Fish". 163-171.

Toxic Substances Control Act No. 139 of 21 December 1994 of the Institute Brasileiro do Meio Ambiente e dos Recursos Naturais Renovaiueis - IBAMA, Brazil.

U.S. Environmental Protection Agency. Pesticide Assessment Guidelines, Series 72, Subdivision E, Hazard Evaluation: Wildlife and Aquatic Organisms.

U.S. Environmental Protection Agency. Standard Evaluation Procedure, Acute Toxicity Test for Freshwater Fish.

ASTM Standard E729-88a. Standard Guide for Conducting Acute Toxicity Tests with Fishes, Macroinvertebrates, and Amphibians.

APPENDIX 1. OUTPUT OF REVIEWER'S STATISTICAL VERIFICATION:

Formulated Product

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
3	.1670867	.1579076	9.472691E-02	.2668476

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
7	.459651	1	.9727836

SLOPE = 5.81926
95 PERCENT CONFIDENCE LIMITS = 1.873943 AND 9.764577

LC50 = .1556398
95 PERCENT CONFIDENCE LIMITS = .1162863 AND .2332496

LC10 = 9.416292E-02
95 PERCENT CONFIDENCE LIMITS = 3.415981E-02 AND .1236109

Zoxamide

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
3	.107972	1.008917E-02	8.15493E-03	1.296685E-02

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
5	2.813252	4.956383	1.932025E-03

SINCE THE PROBABILITY IS LESS THAN 0.05, RESULTS CALCULATED USING THE PROBIT METHOD PROBABLY SHOULD NOT BE USED.

SLOPE = 3.576662
95 PERCENT CONFIDENCE LIMITS =-2.422384 AND 9.575708

LC50 = 9.624541E-03
95 PERCENT CONFIDENCE LIMITS = 0 AND +INFINITY

LC10 = 4.249133E-03
95 PERCENT CONFIDENCE LIMITS = 0 AND +INFINITY

Mancozeb

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
2	.1677541	.1201692	7.558948E-02	.1759518

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
7	.7056288	1	.9995488

SLOPE = 5.499501
95 PERCENT CONFIDENCE LIMITS = .8798256 AND 10.11918

LC50 = .1078416
95 PERCENT CONFIDENCE LIMITS = 7.922898E-02 AND .2599255

LC10 = 6.336596E-02
95 PERCENT CONFIDENCE LIMITS = 6.593066E-03 AND 8.427011E-02